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ORIGINAL PAPER

Neomycin Improves Constipation-Predominant Irritable Bowel Syndrome in a Fashion That Is Dependent on the Presence of Methane Gas: Subanalysis of a Double-Blind Randomized Controlled Study

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Abstract Recent studies have shown that normalization of the lactulose breath test (LBT) with neomycin leads to a significant reduction in irritable bowel syndrome (IBS) symptoms. This subanalysis was done on the constipation-predominant IBS subgroup of patients (C-IBS) to test the ability of neomycin to improve constipation and its correlation with the elimination of methane on breath test. IBS subjects underwent LBT in a blinded fashion. They were then randomly allocated to neomycin or placebo groups. For the purpose of this analysis, only the C-IBS subjects were identified. They were then evaluated for global improvement, abdominal pain, and constipation severity. The ability of neomycin to eliminate methane and its associated improvement in constipation was also determined. One hundred eleven subjects meeting Rome I criteria for IBS were included in the study. Thirty-nine of these had C-IBS. Of these, 20 received placebo and 19 received neomycin. With neomycin, a global improvement of 36.7 ± 7.9% was seen, compared to 5.0 ± 3.2% for placebo ($P < .001$) in the intention-to-treat analysis. Constipation was improved by 32.6 ± 9.9% with neomycin compared to 18.7 ± 7.2% for placebo ($P = .26$). Of the original 111 subjects, 12 demonstrated methane on breath test. All 12 of these patients were constipation predominant. In the methane producers receiving neomycin or placebo, improvement in constipation was significantly greater in those receiving neomycin (44.0 ± 12.3%) compared to placebo (5.0 ± 5.1%) ($P < .05$). Treatment with neomycin improves constipation in C-IBS. This improvement depends on the presence and elimination of methane on breath test.

Keywords Irritable bowel syndrome · Constipation · Methane gas

Introduction

The framework for understanding irritable bowel syndrome (IBS) has changed significantly over the past decade. Some of this change relates to objective findings in IBS such as the influence of serotonin [1]. With the important role of serotonin in peristalsis, drug development has focused on agents that antagonize [2, 3] and stimulate [4] one or more serotonin receptors for the treatment of diarrhea (D-IBS) and constipation-predominant IBS (C-IBS), respectively. As a partial agonist of the serotonin receptor, the prokinetic properties of tegaserod allow this drug to ameliorate symptoms of C-IBS despite no clear evidence that serotonin deficiency is the cause of constipation in this subset of patients.

Another major development in IBS over the last few years has come from the study of gut ecology. One example of this is the now well-recognized phenomenon of postinfectious IBS long after acute gastroenteritis has resolved [5]. The study of postinfectious IBS thus provides an opportunity to observe the development of IBS in a normal host and has been a catalyst for the investigation of gastrointestinal organisms and their role in IBS pathophysiology.

As an extension of this role for enteric bacteria and their influence on IBS development, more recent data also suggest a possible association between small intestinal bacterial overgrowth and IBS [7]. In a series of studies, we have shown that subjects with IBS often have an abnormal lactulose breath test to suggest the presence of bacterial overgrowth

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[7, 8]. These data also reveal that subjects with IBS have a significant improvement in symptoms after receiving neomycin compared to placebo [8] and this improvement is even more significant when neomycin succeeds in normalizing the lactulose breath test abnormality. Corroborating this, up to 12% of IBS patients are now seen to have $>10^6$ coliforms in the most proximal locations of the small bowel such as the duodenum [8]. Furthermore, IBS subjects with culture-proven bacterial overgrowth have more severe bowel complaints when compared to those in whom bacterial overgrowth could not be demonstrated within reach of the upper endoscope [9].

The subgroup of C-IBS constitutes one third of IBS patients [10]. In this subgroup, the concept of bacterial overgrowth is counterintuitive; in traditional thinking, bacterial overgrowth only really factors into the differential diagnosis of diarrhea. However, efforts have recently begun to find a link. Using gas chromatography of human breath samples, enteric organisms produce three major gases, namely carbon dioxide, hydrogen, and methane. Although these gases were thought to be inert, studies have demonstrated an almost doubling of orocecal [11] and whole gut transit time [12] in humans with methane on breath testing. Based on this association with methane, we have now shown that the presence of methane on lactulose breath test is almost universally associated with C-IBS [12]. Follow-up research demonstrates that methane gas itself slows intestinal transit in an in vivo canine model [13].

From these previous data, it appears that methane gas as produced by enteric organisms is not inert and appears to influence intestinal motor activity. Despite these findings, a cause and effect relationship between methane and constipation in humans with IBS has not been established because this would require evaluation of constipation symptoms with the elimination of methane. In this study, we determine the role of methane elimination on constipation in humans with C-IBS to further establish the causative role of this gas.

Methods

Study patients

Subjects with IBS were recruited through advertisement in area newsprint, IBS support groups, and radio with the intention of avoiding recruitment from the tertiary care center. All participants had to meet Rome I criteria [14] for IBS. Subjects were excluded if they had a previous breath test, had taken antibiotics within the last 3 months, had a history of known gastrointestinal disease, or a predisposing factor that was known to lead to bacterial overgrowth (diabetes, narcotic use, active thyroid disease, intestinal surgery [except cholecystectomy], connective tissue disease). The study also excluded subjects with renal insufficiency, hearing impairment, probiotic use, or allergy to aminoglycosides. The study was approved by the Cedars-Sinai Medical Center institutional review board.

Primary study design

Subjects were asked to arrive at the GI Motility Laboratory after avoiding legumes or a heavy meal for dinner and fasting from 7:00 the previous night. After consent, subjects completed a symptom questionnaire using a visual analog scale (VAS) to describe the degree of constipation, diarrhea, abdominal pain, bloating, sense of incomplete evacuation, straining, urgency, mucus, and gas on a 0–5 scale to describe the preceding 7 days.

After an initial breath sample was taken, participants underwent a lactulose breath test. They were given a syrup containing 10 g of lactulose (Inalco, Milan, Italy; packaged by Xactdose, South Beloit, IL). Thereafter, breath samples (end expiratory) were collected every 15 min for 180 min. All breath samples were immediately analyzed for hydrogen and methane and corrected for CO_2 content using a Quintron model SC gas chromatograph (Quintron Instrument Co, Milwaukee, WI). The breath test was then sealed in an envelope to blind the patient and investigators as to the results.

After completion of the blinded breath test, subjects were randomized in double-blind fashion to receive either neomycin 500 mg BID or a matching placebo for 10 days. One week after the completion of the pills, they returned for a repeat questionnaire with VAS score followed by another lactulose breath test as described above following the same guidelines. However, in this follow-up questionnaire, subjects were also asked to rate their global improvement in IBS as a percentage.

As described, at the completion of the study, breath tests were recoded and mixed for blinded interpretation by the reviewer (M.P.). As part of this evaluation, the presence or absence of methane on breath test was determined.

Data analysis

For this subanalysis of this previously reported double-blind study [7], only the C-IBS subjects were evaluated. These subjects were identified based on the VAS scores for constipation and diarrhea. Subjects with C-IBS were those with a baseline constipation score greater than that of diarrhea as previously described [7] on the baseline VAS score. Once C-IBS was identified, the percent global improvement was compared between placebo and neomycin using an intention-to-treat analysis. Secondarily, percent improvement in VAS for constipation and abdominal pain was also determined. The ability of neomycin to eliminate methane was then compared and improvement in constipation

Table 1 Patient demographics between placebo and neomycin among C-IBS subjects

Demographic	Placebo ($n = 20$)	Neomycin ($n = 19$)	P-value
Age	41.2 ± 2.7	45.8 ± 2.3	NS
Gender (F/M)	10/10	6/13	NS
Severity of constipation at baseline	4.0 ± 0.2	4.2 ± 0.2	NS
Number with methane (%)	6 (30)	6 (31.5)	NS
Constipation severity (0–5)	4.0 ± 0.2	4.2 ± 0.2	NS
Abdominal pain (0–5)	3.1 ± 0.4	3.7 ± 0.2	NS

C-IBS constipation-predominant irritable bowel syndrome, *NS* not significant.

with neomycin was compared between those who had and did not have methane on their pretreatment breath test.

Demographic data and outcomes were compared using a Student's t-test with statistical significance set at a $P < .05$. Data were expressed as mean ± standard error. Qualitative data were compared using χ^2.

Results

Among a total of 231 subjects, 111 met Rome I criteria, inclusion criteria, and agreed to participate in the study. Among these subjects, 39 subjects were determined to have C-IBS. Of the 39 C-IBS subjects, 20 received placebo and 19 took neomycin. The demographics of those getting neomycin and placebo were similar (Table 1).

Among the 39 C-IBS subjects, neomycin facilitated a global improvement of 36.7 ± 7.9% compared to 5.0 ± 3.2% for placebo ($P < .001$) using an intention-to-treat analysis (Fig. 1). Out of the 39 subjects, 33 completed all questionnaires and outcomes ($n = 17$ for placebo and $n = 16$ for neomycin). In this group, global improvement was again significantly improved (Table 2). Although constipation and abdominal pain were improved overall, the improvement was not statistically significant (Table 2).

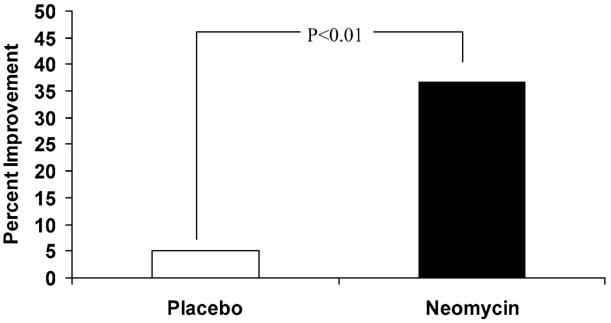

Fig. 1 Comparison of primary endpoint of global improvement in C-IBS patients between neomycin and placebo

Table 2 Percent improvement in global symptoms, constipation, and abdominal pain among patients who completed study

Outcome	Placebo ($n = 17$)	Neomycin ($n = 16$)	P-value
Global Improvement (%)	5.9 ± 3.8	43.6 ± 8.2	<.001
Improvement in constipation (%)	18.7 ± 7.2	32.6 ± 9.9	.26
Improvement in abdominal pain (%)	19.1 ± 7.8	43.3 ± 9.7	.06

The global improvement in C-IBS subjects receiving neomycin was best if the subject had methane on pretreatment breath test. This was evaluated in two ways. First was a comparison between methane and hydrogen producers all of whom had received neomycin. Global improvement was 67.6 ± 5.7% with neomycin in methane producers and 32.7 ± 10.3% in hydrogen producers ($P < .05$) (Fig. 2). Constipation and abdominal pain were not significantly improved in this comparison. The second way was to compare methane producers receiving placebo to those that received neomycin. Out of the 111 original study participants, 12 demonstrated methane on pretreatment breath test. As previously reported, the severity of constipation was 4.1 ± 0.3 in subjects with methane excretion but only 2.3 ± 0.2 in non-methane producers ($P < .01$) and all 12 subjects were among the 30 C-IBS patients [7]. Of the 12 subjects, 10 had complete data ($n = 5$ receiving placebo and $n = 5$ receiving neomycin). When methane producers were analyzed separately, the improvement in constipation was significantly greater among methane producers who received neomycin compared to methane producers receiving placebo (Fig. 3). The improvement in constipation was 44.0 ± 12.3% for those receiving neomycin compared to 5.0 ± 5.1% for methane producers getting placebo ($P < .05$). In fact, the elimination of methane accounted for almost all the improvement in the neomycin category for C-IBS subjects. All five methane-producing C-IBS subjects who

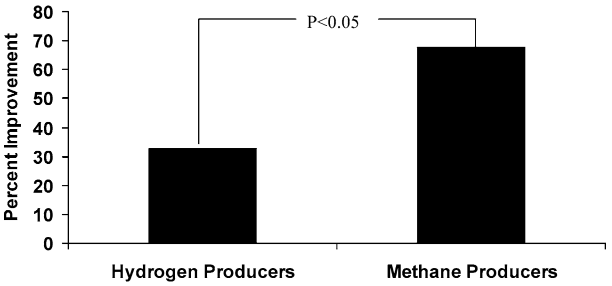

Fig. 2 The global improvement in patients receiving neomycin depends on the baseline gas type

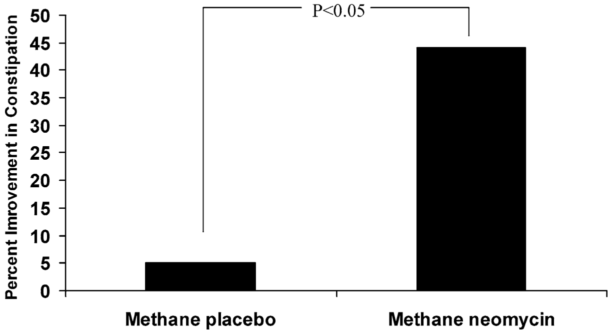

Fig. 3 The role of the initial breath test gas profile on clinical response to constipation. Methane on initial breath test and receipt of antibiotics appears to predict the best outcome in constipation severity

received neomycin eliminated their methane by the follow-up breath test.

Discussion

Previous work has shown that neomycin improves global outcomes in IBS [7]. In this study, we extend the analysis for the subgroup of patients with constipation-predominant symptoms and show that neomycin likewise improves constipation symptoms among C-IBS subjects. First and foremost, the current analysis suggests that patients with C-IBS have a greater overall improvement with neomycin compared to placebo. As in the original study, this improvement appears to depend on the lactulose breath test such that the greatest improvement is seen in subjects whose breath test is improved with neomycin. The difference is that in the C-IBS category, the improvement appears wholly dependent upon the presence and posttreatment elimination of methane on the breath test.

Over the past few years, work has emerged suggesting a role for enteric bacteria in IBS. Much of this discovery relates to the development of IBS after an acute gastrointestinal illness (now referred to as *postinfectious IBS*) [5]. However, work is now suggesting that up to 84% of IBS patients have an abnormal lactulose breath test suggesting the presence of bacterial overgrowth [7]. Although this was a well-conducted double-blind study demonstrating the beneficial effect of neomycin in IBS, critics note specific concerns regarding the link to enteric bacteria. The first is the use of the lactulose breath test, the argument being that breath testing has limitations in clarifying the true location of the bacterial fermentation. Although the breath test is not the gold standard for determining the presence of bacterial overgrowth, small bowel culture using current techniques are far from being a gold standard as well [15]. The limitations of the breath test notwithstanding, the improvement in IBS with antibiotic therapy appears to be linked to the normalization of the test. In other words, 75% improvement in global symptoms is seen when the abnormal breath test is normalized after antibiotics [7], which suggests simply speaking that enteric bacteria appear to play a role in IBS.

Despite the problems with culture, two studies now find that up to 12% of IBS subjects have $>10^6$ coliforms in the upper digestive tract (within reach of an endoscope) [8]. Because these studies only look at the furthest distance from the colon and its coliform contents, such as the duodenum [9], the prevalence would be inevitably higher if more distal small bowel sampling could be conducted.

An obstacle for a bacterial hypothesis in IBS is the heterogeneity of symptoms in IBS. This heterogeneity creates a problem in studying and treating IBS, thus forcing investigators to separate IBS into symptom categories with the hope that this separation will reveal more consistent pathophysiologic findings and consequently treatment options. Thus, criteria for these subtypes are now available [16]. With these criteria, the easiest subgroups to study and define are constipation- and diarrhea-predominant forms of IBS.

Bacterial overgrowth is implicated in IBS symptom production; it is, however, not clear how this excessive enteric bacteria can lead to both constipation and diarrhea. Traditional thinking in bacterial overgrowth is that it manifests as diarrhea [17]. Intriguing data from the original double-blind study with neomycin demonstrate that among the 12 subjects with methane on their lactulose breath test, all were considered to have C-IBS [7]. In addition, they had the most severe constipation among that group. This finding was then corroborated in a second group of subjects that were part of large database [12]. Although these two studies now show that methane appears to be associated with C-IBS, it is far from proving a cause and effect relationship. Some of the considerations are that slow transit in C-IBS creates an environment more suitable for methanogenic proliferation. A second possibility is that the methane gas itself has some role in the slower transit known to occur in C-IBS.

Although the association between the presence of methane and C-IBS is clinical, two previous studies showed that when methane is present on breath testing, orocecal [11] and whole gut [18] transit times are nearly doubled. We conducted two separate experiments to determine whether methane contributed to a change in gut motor function. In the first experiment, methane or room air were infused into the small intestine of a fistulated canine model of transit. In this model, there was universal slowing of transit in every dog with the mean delay of transit of 69% [13].

In a second experiment, a classic guinea pig ileal peristaltic model was used to demonstrate the precise effect of methane on gut motor function. In this organ bath model, methane bubbling of the bath resulted in high-amplitude contraction on both sides of the stimulating brush strokes at an intensity significantly greater than bubbling oxygen alone

[19]. This suggested that the effect of methane might have been to increase contractile response and perhaps this was the cause of slowed transit.

Despite the data suggesting methane causes slow transit in small intestine and the translation of increased motility index in methane-producing IBS subjects, part of the proof for a cause and effect relationship requires a reduction of constipation symptoms as a consequence of eliminating methane on the breath test of these subjects. In the current study, this next step is now shown. The data suggest that neomycin does improve the degree of constipation when compared to placebo in IBS subjects overall. However, among those receiving neomycin, those who had methane on initial breath test not only demonstrated the greatest response, but most of the overall improvement for the neomycin-treated category was related to the elimination of methane on breath test. This provides another piece of evidence that methane may, in fact, be causing the constipation symptoms of C-IBS. To prove this effect finally, transit studies need to be conducted before and after methane elimination on breath test. Transit needs then to be repeated after the return of methane. These studies are ongoing.

A concern with respect to the clinical improvement in C-IBS in this study is that antibiotics are associated with diarrhea in some cases and whether this change in bowel function accounted for the clinical improvement in constipation. This is very unlikely since those subjects treated with neomycin who did not have methane on breath test experienced little or no symptomatic benefit despite being in the neomycin arm of the study.

In conclusion, data are accumulating revealing that methane on lactulose breath test appears to be linked to C-IBS and constipation. The effects may be through slowing of transit by methane as seen in previous study [13]. This study now demonstrates that in humans with C-IBS, the antibiotic neomycin improves constipation in a fashion that depends on the presence and elimination of methane on breath testing.

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